Research Article

Wheat Yield, Physiology and Phenology Response to AM Fungi Application and Phosphorus Management

Mehran Ali* and Inamullah

Department of Agronomy, Faculty of Crop Production Sciences, The University of Agriculture Peshawar, Pakistan.

Abstract | Calcareous soils, particularly having high calcium and carbonate contents reduce Phosphorus (P) solubility and forming complex P compounds, make it unavailable to plants. Inoculation of arbuscular mycorrhizal (AM) fungi could be helpful in the sustainable management of immobile P in soil. However, their use in releasing P from alternative sources in alkaline calcareous soils have been little investigated. To explore the influence of AM fungi and P management on wheat productivity, two years of field experiments were carried out at Agronomy Research Farm, The University of Agriculture Peshawar during Rabi season 2018-19 and 2019-20. Randomized complete block design was used to test the efficacy of different P sources {1. Single super phosphate (SSP), 2. Rock phosphate (RP), 3. Poultry manure (PM), 4. 50% SSP + 50% PM and 5. 50% RP + 50% PM} applied at the rate of 60 and 90 kg P ha^{-1}. These treatments explored with and without incorporation of AM fungi. One control treatment was used for reference. The results exhibited that, AM fungi had non-significant effect on initial phenological stages of wheat like days to emergence and tillering and anthesis but considerable variations were recorded for physiological maturity as well as physiology and yield of wheat crop. Different P levels also revealed the similar trend, 90 kg P ha^{-1} noted better phenology, physiology and yield of wheat, however keeping monetary and sustainability in consideration reduced P level (60 kg ha^{-1}) was more convincing when explored under AM fungi application. Regarding P sources, co-application of SSP and PM in 50:50 ratio, performed comparatively better than the rest of the sources under consideration in field trials. Conclusively, the combined application of SSP and PM at the rate of 60 kg ha^{-1} along with AM fungi incorporation provides an edge over the conventional use of synthetic P fertilizer. Moreover, AM fungi provides improved infrastructure to transfers P to plants for growth promotion under reduced P level, and had more potential to improve wheat yields and P uptake on sustainable basis in P deficient calcareous pH soils.

Received | September 29, 2021; **Accepted** | February 13, 2022; **Published** | September 15, 2023

***Correspondence** | Mehran Ali, Department of Agronomy, Faculty of Crop Production Sciences, The University of Agriculture Peshawar, Pakistan; **Email:** mehran@aup.edu.pk

Citation | Mehran Ali and Inamullah. 2023. Wheat yield, physiology and phenology response to am fungi application and phosphorus management. *Sarhad Journal of Agriculture,* 39(3): 704-715.

DOI | https://dx.doi.org/10.17582/journal.sja/2023/39.3.704.715

Keywords | AM Fungi, Phosphorus Management, Phenology, Physiology, Wheat

Introduction

Wheat (*Triticum aestivum* L.) accounts for the largest source of vegetable protein in human food as well as it fulfills about half of the carbohydrates and one-fifth of the global food calories requirement (FAO, 2020). In Pakistan, it provides nearly 30% to the total food grain basket particularly under cereal-based cropping pattern (MNFSR, 2019). Regardless of immense potential, national average productivity of wheat is 2769 kg ha^{-1}, much lesser than the yield obtained in developed states (MNFSR, 2019). Despite of higher investment and inputs, wheat productivity in Pakistan undergoing stagnation and even follow declining trend in some areas. The declining trend in crop and soil productivity may be attributed to the adoption of conventional practices, imbalance use of fertilizers and no inclusion of soil beneficial biota.

Soil microorganisms particularly arbuscular mycorrhizal (AM) fungi had a major role in wheat-maize cropping system by affecting the efficacy of applied fertilizers and residual influence, therefore lowering the dependency on fertilizers (Hussain *et al*., 2016; Jan *et al*., 2014). Symbiotic associations were made between plants and AM fungi, where up to 90% Phosphorus (P) and 20% Nitrogen (N) of plant supplied from AM fungi with soil hyphal networks in response of plant photosynthates (Bakhshandeh *et al*., 2017). Apart from accessibility, AM fungi increased nutrient interception through hyphae, will possibly reduce nutrient loss from rhizosphere (Cavagnaro *et al*., 2015). Under relatively meager nutrient conditions such associations of symbiosis have been played a pivotal role. For instance, lower availability of soil P could stimulate AM fungi colonization, which ultimately enhance P, N and Zn utilization and avert its losses (Behlr *et al*., 2015). Whereas, intensive agricultural practices including extravagant application of mineral fertilizers, extensive crop-free episodes, improper tillage practices and inclusion of non-synchronized crops have shown adverse effects on population and colonization of AM fungi (Manoharan *et al*., 2017).

P is one of the macro and most important plant nutrients required by crops in large amount for ensuring higher yield (Imran *et al*., 2014) and soil productivity (Inamullah and Khan 2015). P has the mayhem of immobility in the soil and despite of higher level of application only a part of it is available for plant uptake. According to Manimaran (2014), the contribution of phosphorus to biomass production cannot be overlooked. It is involved in many bio-chemical and physiological processes take place in the plants body (Opala *et al*., 2009). In addition to that, another major P contribution is ensuring the crop reproductive growth where it plays a pivotal role in fertilization and translocation of photosynthates from source to sink (Akhtar *et al*., 2016). Pakistani soils are widely P deficient, so its adequate amount application is indispensable for optimum yield and quality of crops (Aslam, 2016). The ever-escalating prices of commercial P fertilizers throughout the country engendering the need to discover some substitute sources and methodology. So, it could give some relief to small scale farmers by lowering production cost and improve the utilization efficiency of applied fertilizers.

Sustainable nutrient management and crop productivity using the available resources, the better option could be co-application of inorganic and organic P sources (Ali *et al*., 2020). Coupling of both organic-inorganic sources may not only improve the crop yield and soil fertility (Sharif *et al*., 2012) but also farmer's net return (Ali *et al*., 2019) because it improves the efficiency of applied fertilizers (Uwah *et al*., 2011) and reduce the fertilizers losses (Zafar *et al*., 2017). Wahid *et al*. (2016) reported 25% higher economic yield with rock phosphate and poultry manure applied in 50:50 ratio. Furthermore, Shahzad *et al*. (2015) documented considerable increase in maize yield and yield related parameters (cob weight, cob length, 100-grains weight, shelling percentage etc.) from their multi-year experiments through application of PM plus mineral phosphatic fertilizers.

Thus, considering the significance of AM fungi and co-application of organic-inorganic P fertilizers, the current research study was designed to study the role of AMF and P fertilizers for getting higher productivity and optimization of wheat phenology and physiology in cereal-based cropping system.

Materials and Methods

AM Fungi

AM Fungi spores were isolated using the Wet-sieving and decanting technique (Wahid *et al*., 2016). This indigenous AMF inoculum was dominated with *Glomus intraradices*, whereas the spores of *Glomus fasiculatum* and *Glomus mossea* were present in minor quantities. For this purpose, 20 g alkaline calcareous soil samples were taken from the field cultivated with spring maize, having silty clay loam rhizosphere. The AMF spores were observed in the soil samples through a binocular microscope having 40X magnification power. For each pot, 100 spores were isolated and stored in Petri plates as suspension at 4°C for about 48 hours prior of application. The suspension was then applied to pots along with sorghum seeds to raise optimum inoculum for application of extensive field trails. The plant roots with rhizosphere soil were applied at the rate of 1 kg m^{-2}.

Experimental site

A series of field trials were conducted at Agronomy Research Farm, The University of Agriculture Peshawar, during Rabi Season 2018-19 and 2019-20. The site of field trial has continental climate and it is located at 71.46^0 E, 34.02^0 N and 359-meter altitude above sea level. Meteorological data obtained from Pakistan Meteorological Department is shown in Figure 1, while the physico-chemical properties of the site are given in Table 1.

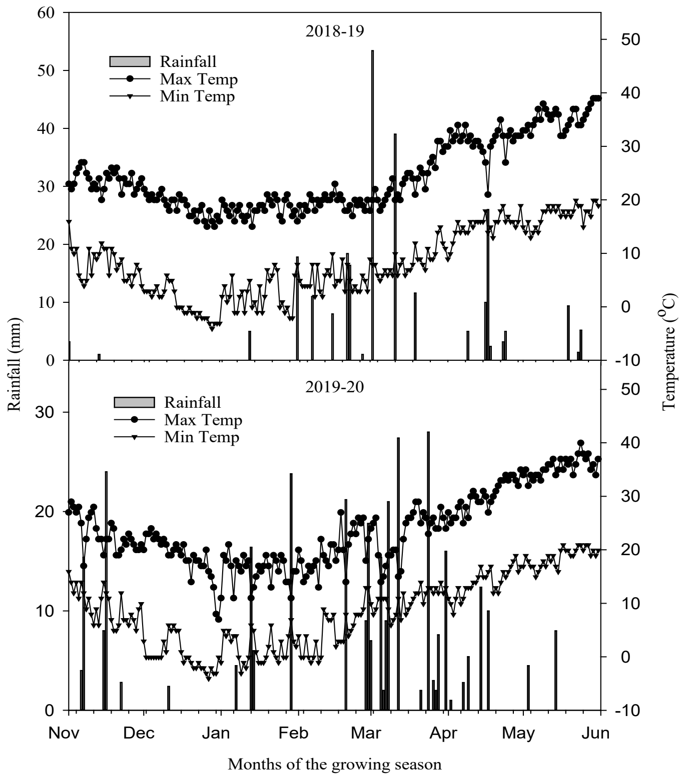

Figure 1: *Maximum and minimum temperatures (°C), and total monthly rainfall (mm) during wheat growing season 2018-19 and 2019-20.*

Experimental treatments and design

Randomized Complete Block design was used to test the efficacy of different P sources {1. Single super phosphate (SSP), 2. Rock phosphate (RP), 3. Poultry manure (PM), 4. 50% SSP + 50% PM and 5. 50% RP + 50% PM} applied at the rate of 60 and 90 kg ha^{-1}. These treatments were explored with and without incorporation of AM fungi. One control treatment was used for reference and the experiment was replicated thrice. Test variety KHAISTA-2017 was planted in a 3m x 3m plot size. Each experimental unit was consisted of ten rows having 0.3m row to row with 3m length of the row. All the P sources were incorporated at the time of sowing along with potash application at the rate of 60 kg ha^{-1} uniformly. However, the half nitrogen (75 kg) was applied at sowing and the remaining half was at tillering stage. Recommended irrigation schedule and other agronomic practices were kept uniform for all the experimental units. The experimental trial was harvested when crop reached harvest maturity *i.e.* 30-34% grain moisture contents, on 11th May in 2020 and 13th May in 2021.

Table 1: *Physico-chemical properties of the experimental site and PM.*

Characteristics	Soil	PM
Sand (%)	7.81	--
Silt (%)	39.4	--
Clay (%)	52.7	--
Textural class	Silty clay loam	
$pH_{1:5}(H_2O)^+$	8.02	7.82
$EC_{1:5}$ (dSm^{-1})$^+$	0.18	1.34
BD (g cm^{-3})	1.25	--
Organic matter (%)	0.84	--
Total Nitrogen (%)	0.051	1.83
Mineral Nitrogen (mg kg^{-1})	19.13	--
Organic carbon (g kg^{-1})	5.73	674
AB-DTPA extractable P (mg kg^{-1})	2.84	25.6
AB-DTPA extractable K (mg kg^{-1})	81.1	--
Calcium carbonate (%)	17.0	--

+ = pH and EC of PM was measured on 1:10 (w/v basis)

Measurements and observations

Germination m^{-2} was considered when 85% seedlings emerged in each experimental unit. Phenological stages were quantified by days' difference between planting to date when about 75% plants in each subplots reached to anthesis and physiological maturity. Similarly, physiological maturity was taken when 70% of the physical structure of the crop stand appeared yellowish-brown. Leaf area index (LAI) was calculated as the ratio of total leaf area (LA) of plants and total ground area covered by the plants. SPAD value was took on 5 flag leaves randomly selected in each plot with SPAD meter to approximate the leaf chlorophyll content. For grain yield, central four rows in each plot were harvested, sundried for couple of days, then threshed, weighed and finally converted to kg ha^{-1} using the formula:

$$\text{Grain yield (kg ha}^{-1}) = \frac{\text{Grain yield of four central rows}}{\text{R} - \text{R distance(m)} \times \text{Row length(m)} \times \text{no. of rows}} \times 10{,}000\text{m}^2$$

Table 2: *Phenological events and germination (m^{-2}) of wheat as affected AMF and P management.*

Arbuscular Mycorrhizal Fungi (AMF)	**Days to emergence**	**Germination (m^{-2})**	**Days to anthesis**	**Days to physiological maturity**
AMF applied	14	120	122	157
AMF not applied	14	120	122	158
LSD ($P<0.05$)	NS	NS	NS	0.42
Phosphorus levels (PL)				
60 kg ha^{-1}	14	118	122	158
90 kg ha^{-1}	14	122	122	157
LSD ($P<0.05$)	NS	NS	NS	0.44
Phosphorus sources (PS)				
Single Super Phosphate (SSP)	14	119	121 c	157 c
Rock Phosphate (RP)	14	117	122 b	158 b
Poultry Manure (PM)	13	123	123 a	159 a
SSP + PM (50:50)	14	123	122 b	159 a
RP + PM (50:50)	14	119	122 b	158 b
LSD ($P<0.05$)	NS	NS	0.57	0.69
Planned mean comparison				
Control	14	114	123	160 a
Rest	14	120	122	158 b
Significance	NS	NS	NS	***
Interactions				
PL x PS	NS	NS	NS	NS
AMF x PL	NS	NS	NS	NS
AMF x PS	NS	NS	NS	NS
AMF x PL x PS	NS	NS	NS	NS

NS: Non-significant; Means within the same category in columns followed by at least one common letter are not significantly different at $P \leq 0.05$ level.

The data was statistically analyzed using the appropriate ANOVA for Randomized Complete block design and LSD at 0.05 level of probability (Jan *et al*., 2009).

Results and Discussion

Crop phenology

Days to emergence and germination m^{-2}: Data regarding days to emergence and germination m^{-2} of wheat as affected by AMF application, P levels and P sources are presented in Table 2. Analysis of the data showed that AMF, P levels and sources had non-significant effect on days taken to emergence and germination counted per unit area. Similarly, planned mean comparison of control against rest had no significant effect on wheat seed emergence interval and seedlings emerged in unit area. Correspondingly, all the possible interactions were also found non-significant.

Days to Anthesis: P sources had significant, while AMF and P levels had non-significant effect on days to anthesis of wheat (Table 2). All the interactions between AMF, P levels and sources were non-significant. Mean values of the data indicated delayed anthesis with application of PM (123 days), followed by RP and RP+PM incorporation. Early anthesis (121 days) was observed in plots where P source was applied from SSP. Control plots in comparison with fertilized plots took more (123 days) to anthesis.

Days to Physiological Maturity: Perusal of the data presented in Table 2, revealed that AMF, P levels and sources had significant effect on days taken to physiological maturity of wheat. The planned mean comparison of control vs rest was also found significant. However, all the possible interactions were found non-significant. Plots incorporated with AMF inoculum noted early physiological maturity (157 days) than no-AMF applied units. Comparing different rates of P, delayed physiological maturity (158 days) noted in P applied at the rate of 60 kg ha^{-1}. Whereas, 90 kg P ha^{-1} applied plots took less days to physiological maturity. Mean values of the different P sources indicated delayed physiological maturity with application of PM (159 days), followed by RP and RP+PM incorporated experimental units. Early physiological maturity (157 days) was observed in plots where SSP was incorporated as a P source. Control plots in comparison with fertilized plots took more (160 days) to physiological maturity.

Table 3: *SPAD value, leaf area index and grain yield (kg ha^{-1}) of wheat as affected AMF and P management.*

Arbuscular Mycorrhizal Fungi (AMF)	**SPAD value**	**Leaf area index**	**Grain yield (kg ha^{-1})**
AMF applied	55	3.84	3875
AMF not applied	53	3.41	3683
LSD (P<0.05)	0.52	0.07	59
Phosphorus levels (PL)			
60 kg ha^{-1}	54	3.55	3631
90 kg ha^{-1}	54	3.69	3928
LSD (P<0.05)	NS	0.08	62
Phosphorus sources (PS)			
Single Super Phosphate (SSP)	54 b	3.67 b	3808 b
Rock Phosphate (RP)	53 c	3.34 c	3616 c
Poultry Manure (PM)	54 b	3.66 b	3743bc
SSP + PM (50:50)	56 a	4.02 a	3968 a
RP + PM (50:50)	54 b	3.42 c	3763bc
LSD (P<0.05)	0.87	0.13	98
Planned mean comparison			
Control	50.1 b	2.48 b	2834 b
Rest	54.2 a	3.62 a	3779 a
Significance	***	***	***
Interactions			
PL x PS	NS	*	**
AMF x PL	NS	**	NS
AMF x PS	*	***	**
AMF x PL x PS	NS	NS	NS

NS: Non-significant; Means within the same category in columns followed by at least one common letter are not significantly different at $P \le 0.05$ level.

Crop physiology

SPAD Value: Data pertaining to SPAD value of wheat as affected by AMF, P levels and P sources are given in Table 3. Statistical analysis of data revealed that SPAD value of wheat differed significantly in response to AMF and different P sources. The difference between control vs rest was significant and indicated that fertilized plots reported higher SPAD value (54.2) than control plots (50.1). However, P levels and all the possible interactions were not significant except AMF x PS. Mean values of AMF application revealed that higher SPAD value (55.0) with the incorporation of mycorrhiza inoculum. Regarding different P sources, co-application of SSP and PM in the 50:50 ratio noted higher SPAD value (55.8) which was statistically similar with PM (54.2) and SSP. Plots fertilized with RP as a P source observed lower SPAD value (53.0) of wheat. In AMF x PS, P applied in the form of both sole and integrated resulted positive increase in wheat SPAD value when AMF is amended. However, exception exists in the case of SSP application, where no considerable increase recorded in SPAD value with AMF (Figure 2).

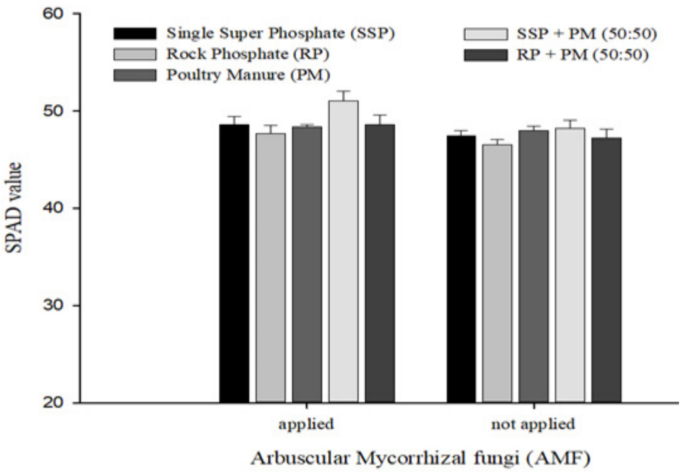

Figure 2: *Interaction between AMF and P sources for SPAD value of wheat.*

Leaf area Index (LAI): Data regarding LAI of wheat as affected by AMF, P levels and P sources are given in Table 3. Statistical analysis of data revealed that LAI of wheat differed significantly in response to application of AMF, P levels and different P sources. The difference between control vs rest was significant and indicated that fertilized plots produced higher LAI compared with control plots. However, all the possible interactions were not significant except PL x PS, AMF x PL and AMF x PS. Mean values of data regarding AMF, indicated higher LAI (3.84) in AMF amended plots than no AMF applied plots (3.41). Likewise, P application at the rate of 90 kg P ha^{-1} produced higher LAI (3.69) as compared with 60 kg P ha^{-1} (3.55). Regarding different P sources, addition of integrated (50%SSP+50%PM) produced higher LAI (4.02), which was followed by SSP amended units with LAI of 3.67. Application of PM and RP+PM produced statistically similar LAI. Plots fertilized with RP produced lower LAI of wheat (3.34). Considering PL x PS interaction effect, significant positive increase observed in LAI when various P sources applied at higher rate than reduced one. At 60 kg P ha^{-1} SSP+PM application showed promising increase compared to rest of sources applied. However, at 90 kg P ha^{-1} highest LAI was observed in sole SSP incorporated plots (Figure 3). The interaction of AMF x PL revealed that AMF incorporation had positive effect on LAI under both levels. However, the effect was more prominent with reduced P application (Figure 4). In AMF x PS, P applied in the form of both sole and integrated responded positively when AMF is amended. However, exception exists in the case of SSP application, where no considerable increase recorded in LAI with AMF incorporation (Figure 5).

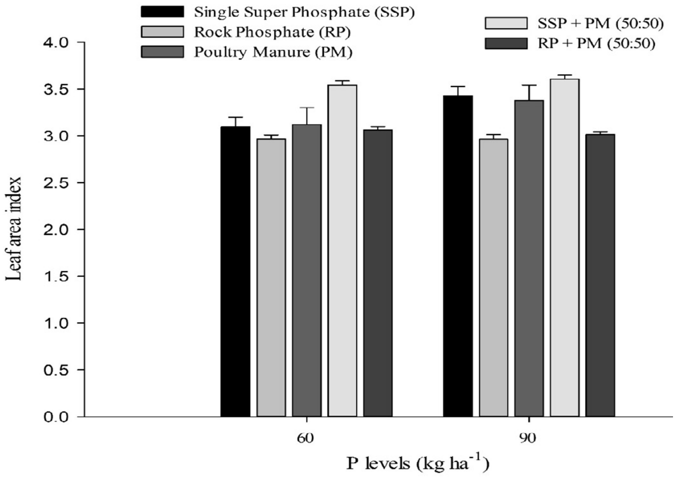

Figure 3: *Interaction between P levels and P sources for leaf area index of wheat.*

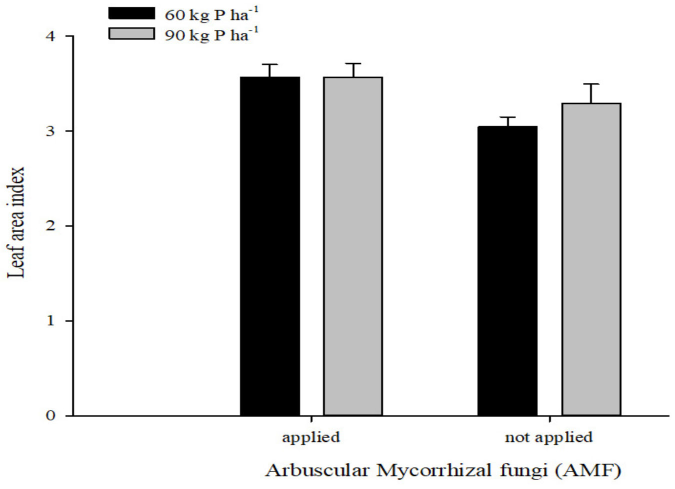

Figure 4: *Interaction between AMF and P levels for leaf area index of wheat.*

Grain Yield (kg ha^{-1})

Analysis of variance showed significant effect of AMF, P levels and various P sources on grain yield of wheat (Table 3). The planned mean comparison of control vs rest was found significant which suggested that fertilized plots resulted higher grain yield (3779 kg ha^{-1}) as compared with control plots (2834 kg ha^{-1}). However, all the possible interactions except PL x PS and AMF x PS were found non-significant for grain yield of wheat. Considering AMF application, plots incorporated with AMF produced higher grain yield (3875 kg ha^{-1}) as compared with no AMF applied plots (3683 kg ha^{-1}). Likewise, P levels also varied the grain yield. Application of 90 kg P ha^{-1} produced higher grain yield (3928 kg ha^{-1}) than reduced P application *i.e.* 60 kg ha^{-1}. Among various P sources, addition of P from SSP+PM produced higher grain yield (3968 kg ha^{-1}) which was followed by SSP. However, grain yield of SSP (3808 kg ha^{-1}), RP+PM (3763 kg ha^{-1}) and PM (3743 kg ha^{-1}) was statistically similar. Application of RP as a P source had lower grain yield (3616 kg ha^{-1}). Interactive response of PL x PS revealed increasing trend in grain yield when the rate of P applied changed from 60 to 90 kg P ha^{-1} irrespective of the sources. 100%SSP and 50%SSP+50%PM showed noticeable increment in grain yield. However, nominal increment reported in the rest of P sources under consideration (Figure 6). In a similar way, different P sources incorporated in combination with AMF, performed significantly better when compared with sole application of them (Figure 7).

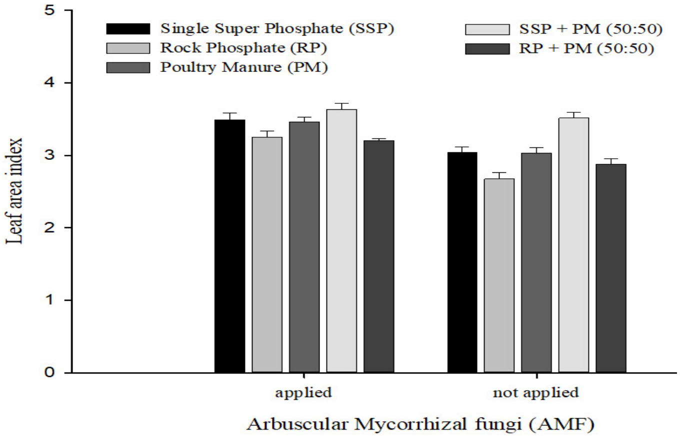

Figure 5: *Interaction between AMF and P sources for leaf area index of wheat.*

AMF incorporation had non-significant effect on earlier events and negatively affects the later phonological observations. The possible reason for the altering crop phenology is P uptake through diffusion process and coupled with increasing root exploration with indigenous AMF addition (Campos *et al*., 2018).

Higher P uptake encourages the reduction of vegetative period and accompanies the transition to reproductive growth (Ortas and Bykova, 2018). Whereas, limiting P acquisition supports delaying the transformation to reproductive stage and it might be dis-proportionately beneficial for P acquirement (Yousefi *et al*., 2011; Nord *et al*., 2011). Similar results were also observed by Pellegrino *et al*. (2015) who documented that wheat growth and phonological observation like days to anthesis and physiological maturity were optimized with AMF inoculation.

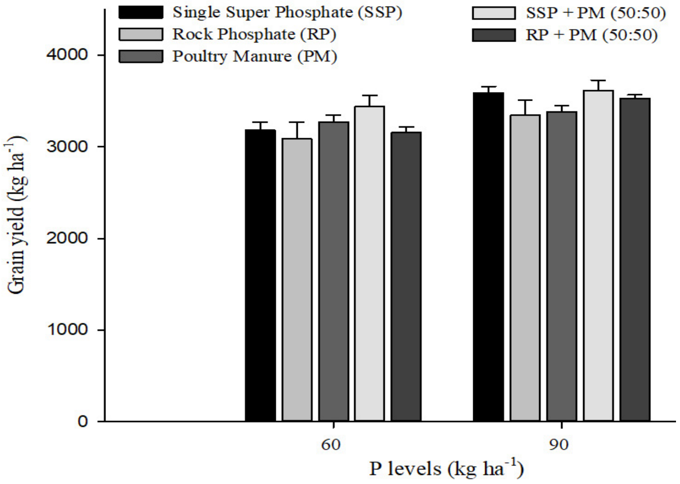

Figure 6: *Interaction between P levels and P sources for grain yield (kg ha^{-1}) of wheat.*

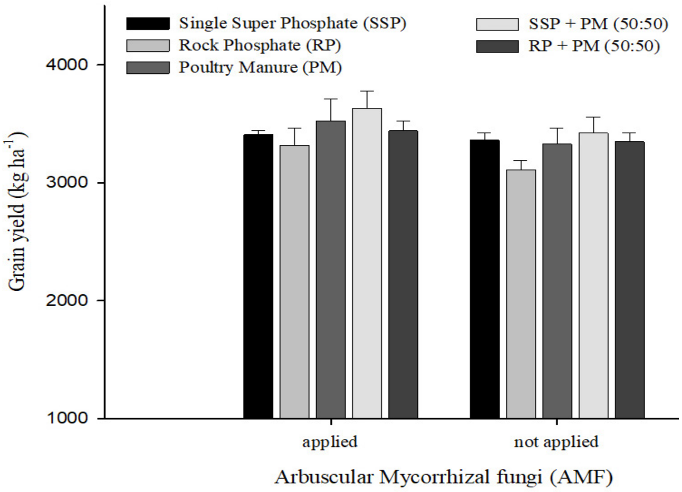

Figure 7: *Interaction between AMF and P sources for grain yield (kg ha^{-1}) of wheat.*

Initial stages like days to emergence and emergence m^{-2} had no considerable variation with applied P irrespective of levels and sources. The emergence of seed mostly depends on above (air temperature) and below (soil temperature) (Saharan *et al*., 2016), food stored inside cotyledon (Saikia *et al*., 2015) and presence of available moisture (Zavattaro *et al*., 2017). Similarly, Shah *et al*. (2009) stated that seed utilizes its stored endospermic food for germination and doesn't need any external food in order to emerge. These results corroborates with Khalil *et al*., (2010) who observed no obvious difference in various P treatments. In contrast later phonological events (days to anthesis and physiological maturity) responded positively to applied P as well as AMF. P application resulted in early flowering and ultimately early maturity. P application was negatively correlated with wheat phenology (Cu *et al*., 2020) due to its role in P uptake. Higher P concentrations in wheat optimize the photosynthetic rate and ultimately grain filling duration in wheat (Cu *et al*., 2020). In addition to that, low P availability typically delays plant phenology (Ma *et al*., 2002). Similarly, Nord and Lynch (2008) documented that one of the plant adaptive responses to low P availability is the delaying of phonological events, to get longer duration for P acquirement and utilization. The results are in accordance with (Hussain *et al*., 2008) who observed early arrival of flowering with increase in P rate eventually curtail the crop life cycle. Likewise, (Dugassa *et al*., 2019; Grant *et al*., 2001) corroborated that under P deficient conditions, wheat delays phonological events, curb primary and secondary root development, and in the long run declines plant canopy and dry matter yield, which are mainly irreversible. Moreover, Ali *et al*. (2019) also confirmed the positive results recorded and reported that plant phenology was optimized with the co-application of organic and inorganic P sources.

AMF incorporation improves wheat physiological parameters than no-AMF applied plots. Addition of AMF increased SPAD value by 2.79%. Likewise, LAI were enhanced by 11.17% in AMF amended plots. AMF have been shown to benefit crop growth and development due to their contribution to plant nutrition, soil structure and other ecosystem services (Ortas and Bykova, 2018). AMF addition can enhance the wheat roots ability to absorb several nutrients, the improvement of nutrients uptake is attributed to the far-reaching and penetrable hyphal and mycelial system. AMF acts as a bridge for nutrient transportation between soil and roots, and hyphae can also assist roots in water uptake (Wahid *et al*., 2016; Ortas 2012b).

Optimum rate of P application plays a vital role in wheat growth. The results revealed that LAI were improved by 3.63% than reduced P level. P is noted especially for enhancement of photosynthetic ability

(Akhtar *et al*., 2015) and conversion of those useful plant compounds, required for optimum development and production (Rafique *et al*., 2018). P deficiency is directly proportional to the discoloration of chlorophyll pigment (Jacob and Lawlor, 1991) and photosynthetic capacity reduction of leaf (Zhu *et al*., 2012). The results corroborate by Dai *et al*. (2016) and Wiens *et al*. (2019) documented that optimum P rate had significant positive impact on wheat physiological parameters. Among different P sources, co-application of SSP and PM in the 50:50 ratio produced higher LAI which was statistically similar with sole SSP applied plots. Similarly, the same treatment resulted taller plants with higher SPAD value. P availability and concentration for plant use is often limited in calcareous soil although it is present in organic as well as in in-organic forms (Wahid *et al*., 2016). The restricted availability is mostly owing to the fixation and complex formation with other nutrients (Shafi *et al*., 2020). So, wisely integration of these available P sources is indispensible for the limited on-farm resources to sustain crop productivity on sustainable basis with lower environmental costs. These results are also in line with the findings reported by Zafar *et al*. (2017), Zhu *et al*. (2012) and Uwah *et al*. (2011) who outlined considerable improvement in physiological parameters which ultimately leads to higher yields under co-application of manures and synthetic fertilizers. Likewise, Pirdashti *et al*. (2010) noted chlorophyll pigments improvement, and Munir *et al*. (2007) reported increase in plant height, leaf area and LAI under integration of P sources.

Soils having high calcium and carbonate contents reduce P solubility and forming complex P compounds (Shafi *et al*., 2020). Field studies showed that AMF incorporation had significant positive impact on yield and yield components. 5% higher grain yield has been recorded with AMF when compared with no-AMF added plots. Higher yield and biomass in AMF amended plots attributed to the protons release and extension of hyphae by AMF for P uptake and acquisition (Smith and Smith, 2011). Confirmatory results are documented by Efthymiou *et al*. (2018) and Smith *et al*. (2015) who found that wheat yield and yield components increased with AMF inoculation under field study. Likewise, Garmendia *et al*. (2017) also confirmed the AMF vitality for sustainable wheat productivity even under a range of environments. A comprehensive study performed by Gupta and Abbott (2020) for AMF inoculated cereals, who documented that AMF inoculation had positive effect on root colonization for essential nutrients uptake and overall wheat productivity (Zhang *et al*., 2019).

In similar manner, 90 kg ha^{-1} P application produced higher grain yield than 60 kg ha^{-1}. As the P application level increased in the present study, all the yield attributes showed positive response. P availability to plants can optimize several physiological processes; photosynthesis, respiration (Noonari *et al*., 2016), storage of energy and cell-division (Bakhsh *et al*., 2008). Similar to our results, Bashir *et al*. (2015) found that yield and yield related attributes had been improved to a certain level of P and reduced level performed better in terms of monetary returns and environmental costs (Xi *et al*., 2016; Reijnders, 2014). In current study comparing various P sources, addition of P from SSP+PM in 50:50 ratio produced higher grain, and biological yield and harvest index which was followed by sole SSP application. Plots fertilized with SSP, SSP+RP and PM was statistically similar for wheat productivity. Similarly, all the yield components observed were higher under the co-application of SSP and PM in 50:50 ratios. Among different P sources under experimental trails, incorporation of RP produced lower yield and yield attributes of wheat in both years. In similar manner, several studies on the integration of organic and synthetic P sources reported noticeable outcomes in cereal-based cropping system than the use of single source (Zafar *et al*., 2017). These additive effects are owing to the fact of gradual release and availability, as P from natural sources have lower susceptibility to loss from rhizosphere (Main *et al*., 2021). The benefits of combined P management were not limited to improving grain yield and its attributes (Venkatesh *et al*., 2019), as on-farm available organic amendments incorporation adds organic matter content to soil which can positively influence soil microbial activity and diversity. Consequently, use of diverse P sources (organic amendments and synthetic fertilizer) along with P solubilizing bio-fertilizers has been encouraged in several occasions (Ali *et al*., 2020; Kaur and Reddy, 2015).

Conclusions and Recommendations

In the light of results and discussion, it is concluded that AMF incorporation improved phenological events and grain yield (3875 kg ha^{-1}) of wheat crop. Likewise, it also had positive impact on SPAD value (55) and LAI (3.84), compared to no-addition of AMF.

P management revealed that co-application of SSP+PM in 50:50 ratio at the rate of 60 kg ha^{-1} reported optimum days to physiological maturity, SPAD value (50), LAI (3.54) and grain yield (3614 kg ha^{-1}). Sole application from SSP at the rate of 90 kg ha^{-1}also performed better and reported statistically similar results for the said parameters.

Based on conclusion, the combined application of SSP and PM at the rate of 60 kg ha^{-1} along with AMF incorporation had more potential to improve wheat yield, phenology and physiology on sustainable basis in P deficient calcareous pH soils.

Acknowledgments

The authors greatly acknowledge Higher Education Commission (HEC) of Pakistan for providing research scholarship and funds under HEC indigenous 5000 PhD Fellowship Program Batch-III, Phase-II with the support of which this research work was successfully conducted.
conducted.

Novelty Statement

This study contributes valuable insights to the field of agricultural management by shedding light on the overlooked potential of AM fungi in mobilizing P from alternative P sources in alkaline calcareous soils. Moreover, it also emphasizes the benefits of combining organic and inorganic P while highlighting the importance of AM fungi for sustainable wheat production and improved P uptake in P-deficient calcareous soils.

Author's Contribution

Mehran Ali: Conducted the field experiment and responsible for all field work from sowing to harvesting and manuscript write-up including statistical analysis, figures and tables development.
Inamullah: Supervisor, helped in the idea, designing and supervision of the research. He shaped and checked the manuscript for publication.

Conflict of interest
The authors have declared no conflict of interest.

References

Akhtar, M., M. Yaqub, A. Naeem, M. Ashraf and V.E.H. Ernandez. 2016. Improving phosphorus uptake and wheat productivity by phosphoric acid application in alkaline calcareous soils. Sci. Food Agric., 96: 3701-3707. https://doi.org/10.1002/jsfa.7555

Ali, M., Inamullah, M. Bilal, S. Ali, F. Nawaz and M.O. Iqbal. 2019. Soil physical properties, total n and maize yield response to various N sources incorporated with different tillage implements. Sarhad J. of Agric., 35(1): 216-224. https://doi.org/10.17582/journal.sja/2019/35.1.216.224

Ali, M., Inamullah, S. Ahmad and A. Khan. 2018. Nitrogen sources incorporation with different tillage implements affects maize productivity and soil organic matter. Sarhad J. Agric., 34(2): 478-485. https://doi.org/10.17582/journal.sja/2018/34.2.478.485

Ali, S., M. Arif, M. Ali, M. Afzaal, G. Saeed, M. Bilal, F. Munsif and S. Zaheer. 2020. Biochar and integrated phosphorus management suppress weed density in maize crop. Pak. J. Weed Sci. Res., 26(4): 491-497. https://doi.org/10.28941/pjwsr.v26i4.911

Aslam, M. 2016. Agricultural productivity current scenario, constraints and future prospects in Pakistan. Sarhad J. Agric., 32(4): 289-303. https://doi.org/10.17582/journal.sja/2016.32.4.289.303

Bakhsh, A., R. Khan, A.R. Gurmani, M.S. Khan, M.S. Nawaz, F. Haq and A. Farid. 2008. Residual/direct of phosphorus application on wheat and rice yield under rice-wheat system. Gomal Uni. J. of Res., 24: 29-35.

Bakhshandeh, S., E. Paola, P. Corneoa, Mariottea, M.A. Kertesza, A. Feike and Dijkstraa. 2017. Effect of crop rotation on mycorrhizal colonization and wheat yield under different fertilizer treatments. Agric. Ecosys. Environ., 247: 130-136. https://doi.org/10.1016/j.agee.2017.06.027

Bashir, S.S. Anwar, B. Ahmad, Q. Sarfraz, W. Khatk and M. Islam. 2015. Response of wheat crop to phosphorus levels and application methods. J. Environ. Earth Sci., 5(9): 151-155.

Behlr. R.K., H. Sharma, V. Kumar and K.P. Singh. 2015. Effect of dual inoculation of VA mycorrhiza and azotobacter chroococcum on above flag leaf characters in wheat. Arch. Agron. Soil

Sci., 79: 29-36.

Campos, P., F. Borie, P. Cornejo, J.A. López-Ráez, A. López-García and A. Seguel. 2018. Phosphorus acquisition efficiency related to root traits: is mycorrhizal symbiosis a key factor to wheat and barley cropping. Front. Plant Sci., 9:752-759. https://doi.org/10.3389/fpls.2018.00752

Cavagnaro, T.R., S.F. Bender, H.R. Asghari and M.G. Van der Heijden. 2015. The role of arbuscular mycorrhizas in reducing soil nutrient loss. Trends Plant Sci., 20: 283–290. https://doi.org/10.1016/j.tplants.2015.03.004

Cu, S.T., G. Guild, A. Nicolson, G. Velu, R. Singhand and J. Stangoulis. 2020. Genetic dissection of zinc, iron, copper, manganese and phosphorus in wheat (*Triticum aestivum* L.) grain and rachis at two developmental stages. Plant Sci., 291:1:12. https://doi.org/10.1016/j.plantsci.2019.110338

Dai, J., Z. Wang, M. Li, G. He, Q. Li, H. Cao. and X. Hui. 2016. Winter wheat grain yield and summer nitrate leaching: Long term effect of nitrogen and phosphorus rates on the Loess Plateau of China. Field Crop Res. 196: 180-190. https://doi.org/10.1016/j.fcr.2016.06.020

Dugassa, A., K. Belete and T. Shimbir. 2019. Response of wheat (*Triticum aestivum* L.) to different rate of nitrogen and phosphorus at Fiche-Salale, Hihglands of Ethiopia. J. Plant Breed. Crop Sci., 6(1): 474-480.

Efthymiou, A., B. Jensenand and I. Jakobsen. 2018. The roles of mycorrhiza and Penicillium inoculants in phosphorus uptake by biochar-amended wheat. Soil Bio. Biochem., 127: 168-177. https://doi.org/10.1016/j.soilbio.2018.09.027

FAO, 2020. Food and Agriculture Organization of the United Nations. FAO. URL http://faostat3.fao.org.(Accessed 23 March 2020).

Garmendia, I., Y. Gogorcena, I. Aranjueloand N. Goicoechea. 2017. Responsiveness of durum wheat to mycorrhizal inoculation under different environmental scenarios. J. of Plant Growth Regulation, 36(4): 855-867. https://doi.org/10.1007/s00344-017-9690-x

Grant, C.A., D.N. Flaten, D.J. Tomasiewicz and S.C. Sheppard. 2001. The importance of early season phosphorus nutrition. Can. J. Plant Sci., 81: 211–224. https://doi.org/10.4141/P00-093

Gupta, M.M. and L.K. Abbott. 2021. Exploring economic assessment of the arbuscular mycorrhizal symbiosis. Symbiosis, 83(2): 143-152.

Hussain, A., P. Kumarand and I. Mehrotra. 2008. Treatment of phenolic wastewater in UASB reactor: effect of nitrogen and phosphorous. Bioresour. Techol., 99(17): 8497-8503. https://doi.org/10.1016/j.biortech.2008.03.059

Hussain, S., M. Sharif, S. Khan, F. Wahid, H. Nihar, W. Ahmad, I. Khan, N. Haider and T. Yaseen. 2016. Vermicompost and mycorrhiza effect on yield and phosphorus uptake of wheat crop. Sarhad J. Agric., 32(4): 372-381. https://doi.org/10.17582/journal.sja/2016.32.4.372.381

Imran, M., M. Arif, S. Ali, S. Ahmad, M. Ullah and M. Habibullah. 2014. Integration of biochar with organic and inorganic sources of phosphorous for improving maize productivity. Integration, 4(11): 211-217.

Inamullah and A.A. Khan. 2015. Plant nutrition. *In*: Agriculture The Basics 3rd ed. pp.164-165.

Jacob, J. and D.W. Lawlor. 1991. Stomatal and mesophyll limitations of photosynthesis in phosphate deficient sunflower, maize and wheat plants. J. Exp. Bot., 42: 1003-1011. https://doi.org/10.1093/jxb/42.8.1003

Jan, B., A. Ali, F. Wahid, S.N.M. Shah, A. Khan and F. Khan. 2014. Effect of Arbuscular Mycorrhiza fungal inoculation with compost on yield and phosphorous uptake of berseem in alkaline calcareous soil. Am. J. Plant Sci., 5: 1359-1369. https://doi.org/10.4236/ajps.2014.59150

Jan, M.T., P. Shah, P.A. Hoolinton, M.J. Khan and Q. Sohail. 2009. Agriculture research: Design and Analysis: A monograph Deptt. of Agron., NWFP Agric. Uni. Peshawar, Pakistan.

Kaur, G. and M.S. Reddy. 2015. Effects of phosphate-solubilizing bacteria, rock phosphate and chemical fertilizers on maize-wheat cropping cycle and economics. Pedosphere, 25: 428-437. https://doi.org/10.1016/S1002-0160(15)30010-2

Khalil, S.K., S. Khan, A. Rahman, A.Z. Khan, I.H. Khalil, I.H., Amanullah, S. Wahab, F. Mohammad, S. Nigar, M. Zubair, S. Parveen and A. Khan. 2010. Seed priming and phosphorus application enhance phenology and dry matter production of wheat. Pak. J. Bot., 42(3):1849-1856.

Khan, A., A. Khan, D.F. Khan, S. Khan, Anjum, W. Ali, H. Akbar and A. Khan. 2021. Optimizing nitrogen sources and tillage practices for wheat crop stand and phenology. Sarhad J. Agric., 37(2): 340-347. https://doi.org/10.17582/journal.sja/2021/37.2.340.347

Ma, K., M. Scheibitz, S. Scholz and M. Wagner. 2002. Applications of boron–nitrogen and boron–phosphorus adducts in organometallic chemistry. J. Organomet. Chem., 652(1-2): 11-19. https://doi.org/10.1016/S0022-328X(02)01303-7

Manimaran, M. 2014. Integrated phosphorus management in maize crop grown in alkaline soil. Int. J. Mod. Sci. Eng. Tech., 1(6): 53-56. https://doi.org/10.1016/j.apsoil.2017.03.012

Manoharan, L., N.P. Rosenstocka, A. Williams and K. Hedlunda. 2017. Agricultural management practices influence AMF diversity and community composition with cascading effects on plant productivity. Appl. Soil Econ. 115:53–59.

Mian, I.A., B. Ahmad, S. Khan, B. Khan, K. Dawar, M. Tariq, M. Mussarat, M.W. Muhammad, S. Ali, H. Bibi and F. Muhammad. 2021. Improving wheat productivity and soil quality through integrated phosphorous management with residual effect of biochar. J. Saudi Chem. Soc., 25(1):101175, 1-9. https://doi.org/10.1016/j.jscs.2020.11.008

MNFSR. 2017. Agricultural Statistics of Pakistan 2017-2018. Ministry of National Food Security and Research (Economic Wing) Government of Pakistan Islamabad.

Munir, M.A., M.A. Malik and M.F. Saleem. 2007. Impact of integration of crop manuring and nitrogen application on growth, yield and quality of spring planted sunflower (*Helianthus annuus* L.). Pak. J. Bot. 39(2): 441-449.

Noonari, Shahzado, S.A. Kalhoro, A. Ali, A. Mahar, S. Raza, M. Ahmed, S.F.A Shah and S.U. Baloch. 2016. Effect of different levels of phosphorus and method of application on the growth and yield of wheat. Nat. Sci., 8 (7): 305-314. https://doi.org/10.4236/ns.2016.87035

Nord, E.A. and J.P. Lynch. 2008. Delayed reproduction in Arabidopsis thaliana improves fitness in soil with suboptimal phosphorus availability. Plant Cell Environ., 31(10): 1432-1441. https://doi.org/10.1111/j.1365-3040.2008.01857.x

Nord, E.A., K. Shea and J.P. Lynch. 2011. Optimizing reproductive phenology in a two-resource world: a dynamic allocation model of plant growth predicts later reproduction in phosphorus-limited plants. Ann. Bot., 108(2): 391-404. https://doi.org/10.1093/aob/mcr143

Opala, P.A., C.O. Othieno, J.R. Okalebo and P.O. Kisinyo. 2009. Effect of combining organic materials with inorganic phosphorus sources on maize yield and financial benefits in Western Kenya. Exp. Agric., 46: 23–34. https://doi.org/10.1017/S0014479709990457

Ortas, I. 2012. The effect of mycorrhizal fungal inoculation on plant yield, nutrient uptake and inoculation effectiveness under long-term field conditions. Field Crops Res., 125:35–48. https://doi.org/10.1016/j.fcr.2011.08.005

Ortas, I. and A. Bykova. 2018. The effect of mycorrhiza inoculationand phosphorus application on phosphorus efficiency of wheat plants. Comm. in Soil Sci. Plant Anal., 49(1):1-10. https://doi.org/10.1080/00103624.2018.1455849

Pellegrino, Elisa, M. Öpik, E. Bonari and L. Ercoli. 2015. Responses of wheat to arbuscular mycorrhizal fungi: a meta-analysis of field studies from 1975 to 2013. Soil Biol. Biochem., 84: 210-217. https://doi.org/10.1016/j.soilbio.2015.02.020

Pirdashti, H., A. Motaghian and M.A. Bahamanyar. 2010. Effects of organic amendments application on grain yield, leaf chlorophyll content and some morphological characteristics in soybean cultivars. J. Plant Nutr., 33(4): 485-495.

Rafique, R., Z. Zahra, N. Virk, M. Shahid, E. Pinelli, J. Kallerhoff, T.J. Park and M. Arshad. 2018. Data on rhizosphere pH, phosphorus uptake and wheat growth responses upon TiO2 nanoparticles application. Data in brief, 17: 890-896. https://doi.org/10.1016/j.dib.2018.02.002

Reijnders, L. 2014. Phosphorus resources, their depletion and conservation: A review. Resour. Conserv. Recycl., 93: 32–49. https://doi.org/10.1016/j.resconrec.2014.09.006

Saharan, V., R. Kumaraswamy, R.C. Choudhary, S. Kumari, A. Pal, R. Raliya and P. Biswas. 2016. Cu-Chitosan nanoparticle mediated sustainable approach to enhance seedling growth in maize by mobilizing reserved food. J. Agric. Food Chem., 64: 6148-6155. https://doi.org/10.1021/acs.jafc.6b02239

Saikia, P., S.S. Bhattacharya and K.K. Baruah. 2015. Organic substitution in fertilizer schedule: Impacts on soil health, photosynthetic efficiency, yield and assimilation in wheat grown in alluvial soil. Agric. Ecosyst. Environ. 203: 102-109. https://doi.org/10.1016/j.agee.2015.02.003

Shafi, M.I., M. Adnan, S. Fahad, F. Wahid, A. Khan, Z. Yue, S. Danish, M. Zafar-ul-Hye, M. Brtnicky and R. Datta. 2020. Application of single superphosphate with humic acid improves the growth, yield and phosphorus uptake of wheat (*Triticum aestivum* L.) in calcareous soil. Agron., 10(9): 1224, 1-15. https://doi.org/10.3390/agronomy10091224

Shah, M.M., Q. Khalid, U.W. Khan, S.A.H. Shah, S.H. Shah, A. Hassan and A. Pervez. 2009. Variation in genotypic responses and biochemical analysis of callus induction in cultivated wheat. Genet. Mol. Res., 8(3): 783-793.

Shahzad, K., A. Khan, J.U. Smith, M. Saeed, S. A. Khan and S.M. Khan. 2015. Residual effects of different tillage systems, bioslurry and poultry manure on soil properties and subsequent wheat productivity under humid subtropical conditions of Pakistan. Intl. J. Biosci., 6(11): 99-108. https://doi.org/10.12692/ijb/6.11.99-108

Sharif, M., S. Saud, T. Burni, M. Afzal, F. Khan, M.J. Khan and F. Wahid. 2012. Effect of arbuscular mycorrhizal fungal inoculation in combination with different organic fertilizers on maize crop in eroded soils. Pak. J. Bot., 44(4): 1427-1432.

Smith, S.E. and F.A. Smith. 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth. New paradigms from cellular to ecosystem scales. Annu. Rev. Plant Biol., 62: 227–250. https://doi.org/10.1146/annurev-arplant-042110-103846

Smith, S.E., M. Manjarrez, R. Stonor, A. McNeill and F.A. Smith. 2015. Indigenous arbuscular mycorrhizal (AM) fungi contribute to wheat phosphate uptake in a semi-arid field environment, shown by tracking with radioactive phosphorus. Appl. Soil Econ., 96: 68-74. https://doi.org/10.1016/j.apsoil.2015.07.002

Uwah, D.F., F.A. Afonne and A.R. Essien. 2011. Integrated nutrient management for sweet maize (*Zea mays* L.) saccharata Strut.) production in calabar, Nigeria. Aus. J. Basic App. Sci., 5(11): 1019-1025.

Vanlauwe, B., J. Kihara, P. Chivenge, P. Pypers, R. Coe and J. Six. 2011. Agronomic use efficiency of N fertilizer in maize-based systems in sub-Saharan Africa within the context of integrated soil fertility management. Plant Soil, 339(1–2): 35–50. https://doi.org/10.1007/s11104-010-0462-7

Wahid, F., M. Sharif, S. Steinkillner, M.A. Khan and K.B. Marwat. 2016. Inoculation of Arbuscular mycorrhizal fungi in presence of rock phosphate improve phosphorus uptake and growth of maize. Pak. J. Bot., 48(2): 739-747.

Wahid, F., S. Fahad, S. Danish, M. Adnan, Z. Yue, S. Saud, M.H. Siddiqui, M. Brtnicky, T. Hammerschmiedt and R. Datta. 2020. Sustainable management with mycorrhizae and phosphate solubilizing bacteria for enhanced phosphorus uptake in calcareous soils. Agric., 10(8): 1-14. https://doi.org/10.3390/agriculture10080334

Wiens, J.T., B.J. Cade-Menun, B. Weiseth, and J.J. Schoenau. 2019. Potential phosphorus export in snowmelt as influenced by fertilizer placement method in the Canadian Prairies. J. Environ. Quality, 48(3): 586-593. https://doi.org/10.2134/jeq2018.07.0276

Xi, Bin., L. Zhai, J. Liu, S. Liu, H. Wang, C. Luo, T. Ren and H. Liu. 2016. Long-term phosphorus accumulation and agronomic and environmental critical phosphorus levels in HaplicLuvisol soil, northern China. J. Integ. Agric. 15(1): 200-208. https://doi.org/10.1016/S2095-3119(14)60947-3

Yousefi, A.A., K. Khavazi, A.A. Moezi, F. Rejali and H.A. Nadian. 2011. Phosphate solubilizing bacteria and arbuscular mycorrhizal fungi impacts on inorganic phosphorus fractions and wheat growth. World Appl. Sci. J., 15 (9): 1310-1318.

Zafar, M., M.S. Rizwan and M. Shahid. 2017. Introduction of composted rock phosphate and poultry manure enhances winter wheat phosphorus use efficiency, grain yield and soil quality. J. Plant Nut., 40(13): 1887-1899. https://doi.org/10.1080/01904167.2016.1270316

Zavattaro, L., L. Bechini, C. Grignani, F.K. van Evert, J. Mallast, H. Spiegel, T. Sanden, A. Pecio, J.V. GiraldezCervera, G. Guzman, K. Vanderlinden, T. D'Hose, G. Ruysschaert and H.F.M. Ten Berge. 2017. Agronomic effects of bovine manure: A review of long-term European field experiments. Eur. J. Agron., 90: 127-138. https://doi.org/10.1016/j.eja.2017.07.010

Zhang, S., A. Lehmann, W. Zheng, Z. You and M.C. Rillig. 2019. Arbuscular mycorrhizal fungi increase grain yields: A meta-analysis. New Phytol., 222(1): 543–555.

Zhu, X., C. Li, Z. Jiang, L. Huang, C. Feng, W. Guoand and Y. Peng. 2012. Responses of phosphorus use efficiency, grain yield, and quality to phosphorus application amount of weak-gluten wheat. J. Integ. Agric., 11(7): 1103-1110. https://doi.org/10.1016/S2095-3119(12)60103-8